Composition of Maple Sap and Sirup
Some Aromatic Compounds in Sap

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SUMMARY

The constituents of a chloroform extract of maple sap were resolved by gas chromatography. The high-boiling components were found to be the aromatic compounds coumarin, vanillin, syringaldehyde, coniferyl aldehyde, and 2,6-dimethoxybenzoquinone. None were present in the sap in concentrations greater than 1 ppm. An ether-insoluble lignin was indicated by both chemical tests and infrared data. No significant differences were observed between the chromatograms of early and late sap extracts.

INTRODUCTION

In earlier publications the authors reported the presence of vanillin, syringaldehyde, and dihydroconiferyl alcohol in maple sirup. This initiated an investigation of the maple sap from which the sirup is made to find possible precursors of these compounds or other sap constituents that could be precursors of maple flavor. Skazin (1930) reported that the characteristic flavor of maple sirup and sugar is not found in the sap, but is developed during the atmospheric boiling process used to concentrate the sap to sirup. Experimental work in the authors' laboratory has substantiated this. Findlay and Snell (1935) had confirmed this, but could find no definite flavor precursor in the sap. Risi and Labrie (1935) also failed to find maple precursors in the sap during a series of studies on the production of maple sirup and sugar. The present paper reports the isolation and identification of aromatic compounds in sap of the maple tree that could be related to flavor components in the sirup.

EXPERIMENTAL

Extraction of the sap. As in the work on the flavor components of maple sirup, chloroform was used to obtain from sap an extract free of sugars and color. The trace amounts of compounds to be isolated necessitated that a large quantity of sap be extracted. Also, to minimize the chemical changes induced in it by microorganisms the sap had to be extracted immediately after exuding from the tree. Consequently, a special apparatus for extracting a large volume of sap was designed and installed in a sugar bush during the maple season. Two thousand gallons of sap from 300 trees was collected and transported by a system of plastic tubing to a tank and then into the extractor. The tank provided the needed constant pressure to cause the sap to flow through the extractor at a uniform rate. The extractor consisted of a galvanized steel cylinder 36 inches high and 10 inches in diameter, with a funnel-shaped bottom. The cylinder was half-filled with porcelain "saddles," and five gallons of ACS grade of chloroform was added. Sap from the holding tank entered the bottom of the extractor and bubled up through the chloroform and out a spout at the top. Since $CHCl_3$ is very slightly soluble in water, the sap in passing through the solvent gradually reduced its volume. Therefore, when the five-gallon batch of $CHCl_3$ in the extractor was reduced to 2.5 gallons, the solvent was removed and a new five-gallon lot of $CHCl_3$ added. It was possible to extract 500 gallons of sap per five-gallon charge of $CHCl_3$. Four such 2.5-gallon lots of extract were obtained during the 1962 maple season, representing early-, mid-, and late-season sap. These four lots of $CHCl_3$ were then brought to the laboratory and concentrated to 25 ml each by allowing them to stand at room temperature in a draft of air in a hood. The four lots were then held in glass-stoppered bottles for chemical and gas chromatographis examination.

Gas-liquid chromatographic (GLC) separations. The equipment and conditions for the gas chromatographic fractionation of the $CHCl_3$ extract of

[a] Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

Table 1. Gas chromatographic operating conditions.

Model	Aerograph A-350 (with dual columns and thermal conductivity detectors)
Columns	Stainless steel, ¼″ O.D., 4′ long
Packing	20% Carbowax 20M on 60/80 mesh acid washed Chromosorb W
Temperatures	Injector—270°C Columns—90–240°C Detector—280°C
Flow Rate	100 ml He/min

maple sirup described by Underwood and Filipic (1963) were found satisfactory for the sap extract and are listed in Table 1. The Aerograph A350, using columns packed with either Carbowax 20M or SE-30, gave good separations, the Carbowax being the better of the two. A typical chromatogram resulting from the injection of 500 μl of the concentrated sap extract is shown in Fig. 1. The column was initially maintained 10 min at 90° after injection of sample to allow complete elution of the very large solvent peak, and the temperature was then programmed at the rate of 4° per min to 240°C, at which setting it was held until the last peak was obtained (about 65 min).

Since there was a possibility that some of the peaks obtained were due to the high injection temperature, the following experiment was performed. A 1-ml portion of the concentrated extract was sealed in a thick-walled glass tube under nitrogen. The tube was inserted into a Carius apparatus, brought to 300°C in about 4 hr and then allowed to cool to room temperature. A chromatogram obtained using 500 μl of this heated extract was compared to that from the unheated sample. Fractions whose peak heights increased significantly were considered possible artifacts resulting from the high injection temperature. These peaks are labeled "A" in Fig. 1.

RESULTS AND DISCUSSION

GLC isolates. Attention was focused on the higher-boiling compounds (peaks 7–13) since they were easier to isolate for physical identification. As shown in Table 2, diethyl phthalate, coumarin, vanillin, dibutyl phthalate, syringaldehyde, coniferyl aldehyde, dioctyl phthalate, and 2,6-dimethoxybenzoquinone were present in this extract of maple sap. These identifications, based on infrared spectra, were confirmed by cochromatography of the individual fraction with the indicated compound on a polar (Carbowax 20M) and comparison of retention times on a nonpolar (SE-52) column. Concentrations of these compounds were estimated to be less than 1 ppm, based on comparison of peak areas with standards of known concentration. In the case of vanillin, the detection of the characteristic odor plus the fact that its presence in sirup had been established, strengthened its identification. The coumarin fraction also exhibited its characteristic bitter taste and sweet odor, while the quinone fraction consisted of orange crystals of metallic luster which dissolved to give a strong yellow color in chloroform. The vanillin and coumarin

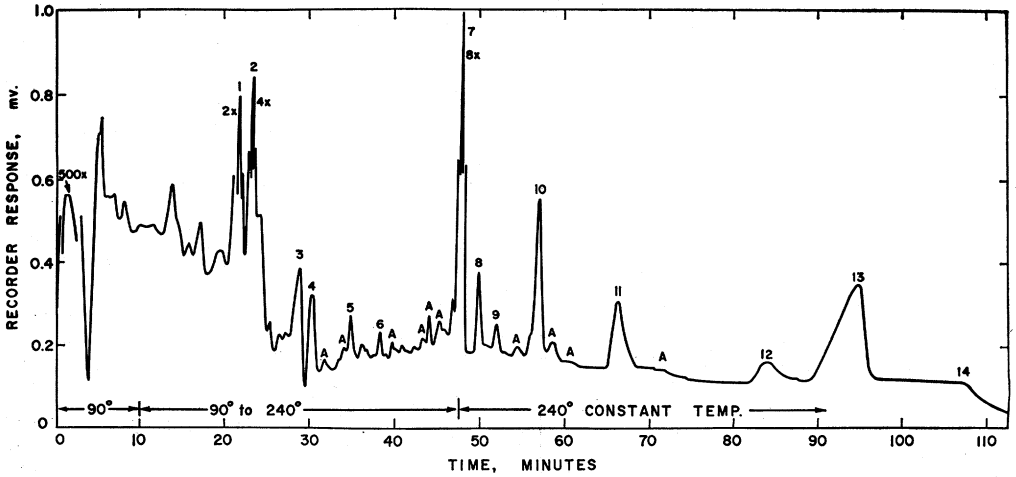

Fig. 1. A gas chromatogram of the chloroform extract of maple sap (on Carbowax 20M).

Table 2. Identification of maple sap components.

Peak no.	Identity	Evidence		
7	Diethyl phthalate	R.T.,[a]	Co.,[b]	I.R.[c]
8	Coumarin	R.T.,	Co.,	I.R., Odor[d]
9	Vanillin	R.T.,	Co.,	I.R., Odor
10	Dibutyl phthalate	R.T.,	Co.,	I.R.
11	Syringaldehyde	R.T.,	Co.,	I.R.
12	Coniferyl aldehyde	R.T.,	Co.,	I.R.
13	Dioctyl phthalate	R.T.,	Co.,	I.R.
14	2,6-Dimethoxybenzoquinone	I.R.,	Color[e]	

[a] Retention time of the collected fraction equal to that of a known on an SE-52 column.
[b] Cochromatographed on a Carbowax 20M column.
[c] Comparison of spectra with authentic samples.
[d] Characteristic odor detected.
[e] Intense color of solid and chloroform solution noted.

found in the sap could contribute to the flavor of the sirup. Of the other GLC isolates, coniferyl aldehyde oxidizes readily to vanillin, and so can be considered a possible flavor precursor.

Peaks 7, 10, 13 (Fig. 1) have been respectively identified as diethyl, dibutyl, and dioctyl phthalate esters. Such compounds are commonly used plasticizers and may have been "dissolved" by the sap while in the plastic tubing collection lines. To determine whether this could be the case a $CHCl_3$ extract was obtained from sap that had not been in contact with plastic tubings that might contain phthalate esters. No dioctyl phthalate was noted in this extract. However, two fractions did cochromatograph with added diethyl and dibutyl phthalate, but not enough material was available for infrared absorption data. In spite of this evidence to the contrary the authors feel that these two phthalates are also contaminants of unknown origin.

All fractions from the Carbowax column collected above 200°C were contaminated by substrate bleed. This contamination was so great that it was not possible to obtain good infrared curves. Therefore, these fractions were individually redissolved in $CHCl_3$ and rechromatographed on an SE-52 column. Pure fractions resulted, which gave much improved infrared curves.

The amounts collected, however, were in the submilligram range and required special techniques for infrared analysis. The fractions identified as coumarin and coniferyl aldehyde were run in carbon disulfide solution in ultramicro "D" cells (0.05 mm path length) (Connecticut Instrument Company, Wilton, Connecticut). Chloroform solutions of the vanillin and 2,6-dimethoxybenzoquinone fractions were evaporated to dryness in the cavity of the cell. The location of the infrared bands for the latter fraction agreed with those reported by Flaig and Salfeld (1959). Also, the quinone prepared from 2,6-dimethoxyphenol by the method of Davidge *et al.* (1958) gave an identical spectrum. Syringaldehyde was present in the greatest concentration, and the amount isolated was sufficient to coat (by evaporation from chloroform solution) a specially designed micro salt window of greater area than the "D" cell. The detailed spectrum obtained was completely identical with that of a standard. In each case, the infrared spectrum of the fraction was compared with that of a known compound treated in the same manner. Work is being pursued on identification of the constituents of lower retention time, representing the more volatile compounds in the extract.

Lignin component. The $CHCl_3$ extracts of sap gave the classical Weisner phloroglucinol-HCl reaction for lignin, but lignin was not detected in any fraction separated by GLC. Therefore, an effort was made to isolate this Weisner-positive component by chemical means. The addition of four volumes of diethyl ether to the $CHCl_3$ extract precipitated a fluffy white material. This was isolated by filtration through sintered glass, but upon drying it turned a light brown and was only partially resoluble in $CHCl_3$. The material precipitated by ether

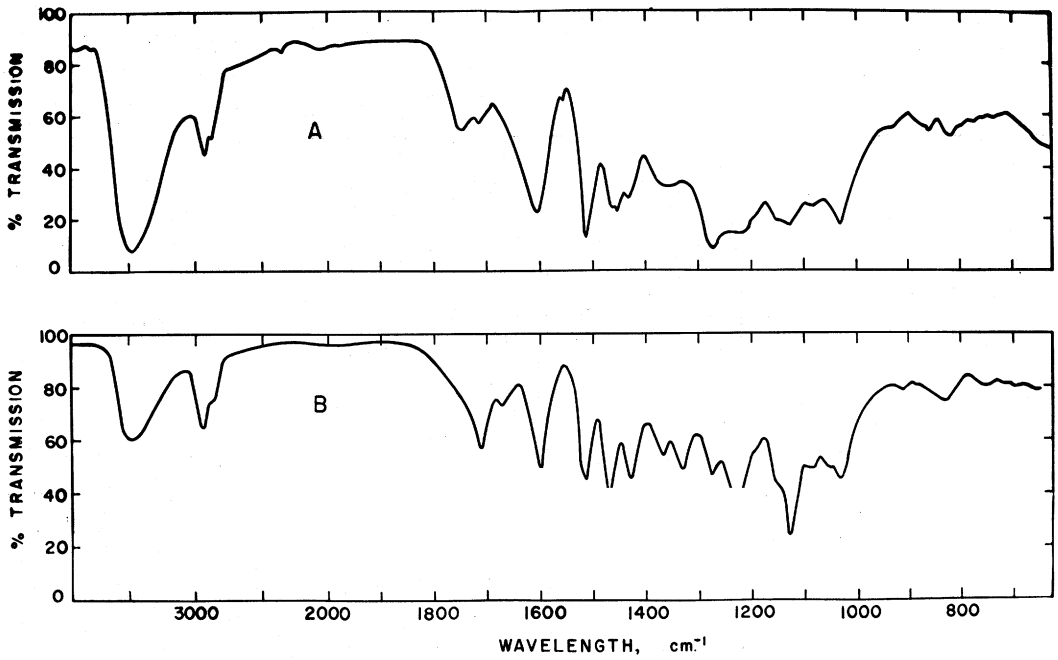

Fig. 2. A comparison of the infrared absorption curves of isolated lignins. A) Native lignin of western hemlock isolated by Hergert; B) Lignin from maple sap.

was therefore separated by centrifugation. The precipitate, when redissolved in $CHCl_3$, gave a strong phloroglucinol-HCl reaction whereas the ether-chloroform soluble fraction gave only a faintly positive reaction. This positive reaction of the ether-chloroform solubles could be due to either a small amount of lignin not removed by this procedure or to the minute quantity of coniferyl aldehyde present in the solution. The infrared spectrum of this precipitate was similar in many respects to those of lignin preparations by Kudzin *et al.* (1951) and by Kolboe and Ellefsen (1962).

The spectrum obtained for native lignin by Hergert (1961) is compared with that obtained by the authors (Fig. 2). Findlay and Snell (1935) reported the possible presence of such material in maple sap based on chemical color reactions alone. This more specific infrared evidence has confirmed their finding. Vanillin, syringaldehyde, and dihydroconiferyl alcohol are well known degradation products of lignin. The relationship of the lignin component as the possible precursor to these and other aromatic compounds in sap and sirup and to the as yet unidentified maple flavor constituents of sirup, will be pursued in further studies.

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